OPTIC NERVE ASSESSMENT IDENTIFIES BIOMARKERS OF AXONAL PATHOLOGY IN MULTIPLE SCLEROSIS

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ABSTRACT

Our aim was to identify possible correlations between physical disabilities assessed by expanded disability status score (EDSS) and neurodegenerative process measured by retinal nerve fibers thickness, and also with demyelization process measured by visual evoked potential with P100 wave latency at optic nerve level on patients diagnosed with multiple sclerosis (MS).

This study aimed to find a structural biomarker at central nerve system (CNS) level which could correlate with clinical disability. The optic nerve is an accessible structure of CNS easy to investigate.

We analyzed a population of 111 patients diagnosed with different clinical forms of multiple sclerosis based on 2005 revised Mc Donald criteria, for whom we assessed clinical disability by EDSS, and by different subscales of multiple sclerosis composite (MSFC) as timed to walk 25 foot (TW25F) and nine hole peg test (9HPT).

Optic nerve involvement was assessed for each eye measuring visual acuity (VA) (Snellen chart), P100 wave latency by visual evoked potential (VEP) and retinal nerve fiber layer thickness (RNFL) by optical coherence tomography (OCT).

The medium age of our population was 38.09 and the medium EDSS was 3.3. – 76 out of 111 were women. 79 patients were diagnosed with relapsing remitting (RR) MS, 7 with clinically isolated syndrome (CIS), the other were diagnosed with progressive form of MS: 7 with primary progressive MS and one with primary progressive with relapse MS.

We obtained positive correlations, and statistically significant between EDSS and 9HPT for dominant hand ($r = 0.58$, $p = 0.0001$) and also for non dominant hand ($r = 0.66$, $p = 0.0001$) using Pearson correlation.

EDSS score correlates statistically significant with P100 wave latency and visual acuity for both eyes using Pearson correlation, so we obtained negative correlation between EDSS and VA for right eye ($r = -0.45$, $p = 0.0001$), and for left eye ($r = -0.49$, $p = 0.0001$).

We also observed statistically significant positive correlation between EDSS and P100 wave latency for right eye ($r = 0.405$, $p = 0.0001$), and left eye ($r = 0.400$, $p = 0.0001$).

RNFL doesn't correlate with EDSS, VA or P100 wave latency. Using chi square correlation between EDSS score (we choose a cut of value of 3) and pathological value of RNFL by OCT we didn't obtain a statistical significant result. The results were : for right eye phi = 0.01, p = 0.9), and for left eye (phi = -0.1, p = 0.3). Similar results were obtained for EDSS of 4.0. The results were: for right eye (phi=-0.009, p= 0.9), and for left eye (phi = 0.017, p = 0.8).

In conclusion, in order to understand the pathology of multiple sclerosis the anterior optic nerve pathway offers an attractive model. Optic nerve assessment can be realized through non-invasive complementary methods. Functional and structural data of optic nerve can be obtained by VA measurement, VEP and OCT assessments.

Our study showed that axonal pathology of the optic nerve (part of CNS exclusively composed by white matter) correlates with clinical disability measured by EDSS, suggesting that P100 latency is a functional biomarker of the disease. A prospective study to validate such a marker of the progression of the disease is needed.

Key words: optic nerve, axonal pathology, multiple sclerosis

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BACKGROUND

Multiple sclerosis is the most frequent neurological disease among young Caucasian population (onset usually between ages 20-40). It is a chronic disease of the central nervous system, characterized by episodes of inflammation and focal demyelination (disseminated in time) and continuous axonal loss. The disease has an immune mechanism (attack against the myelin proteins mediated by T cells) associated with a degenerative process with abnormal *oligodentrocite* function, with axonal degeneration (parallel process) (1,2,3).

Optic nerve, as part of the central nervous system, is frequently impaired during relapsing episodes of multiple sclerosis (neurological focal deficiencies that can be clinically assessed in case of optic nerve impairment as optic neuritis). The process is basically a demyelinating one, but the nerve also suffers a degenerative axonal process that occurs from the precocious states of the disease. It has not been clearly assessed so far if this is a secondary process to demyelination or if it is a primary process, coexistent with demyelination. (3,4).

At the optic nerve level, the demyelination process measurement can be assessed by the latency of P100 wave of the visual evoked potential (electrophysiological method for optical pathway investigation). The value is prolonged, respectively abnormal, if it exceeds 120 milliseconds.

The axonal degeneration of the optic nerve may be evaluated by the thickness of the retinal fiber layer assessed by retinal optical coherence tomography (OCT).

From a clinical point of view, the multiple sclerosis patients are being evaluated by the aid of Kurtzhe's EDSS scale (Expanded Disability Status Scale). This method quantifies the neurological disability on a scale from 0 to 10, where 0 = neurological exam within normal limits and 10 = death by multiple sclerosis. An intermediate score of 7 reffers to a patient restricted to a wheelchair, who cannot walk for 5 meters even with help.

Depending of the clinical evolution of the disease, 4 types of multiple sclerosis may be defined: relapsing remitting, secondary progressive, primary progressive and primary progressive with relapse. The imaging and biologically suggestive onset is defined as clinically isolated syndrome (5).

In over 85% of the cases, the disease begins with attacks (the relapsing remitting type), and over 90% of these cases pass into a progressive type with slowly progressing neurological deficiencies (the secondary progressive type) (6).

PURPOSE OF THE STUDY

Our goal was to identify possible correlation between the physical disability assessed by Expanded Disability Status Score (EDSS) and the degree of axonal degeneration measured at the level of optic nerve (thickness of the retinal fiber layer), but also with the degree of demyelination of the optic nerve (visual evoked potential with measurement of P100 wave latency).

The study aims to identify a structural biomarker at central nervous system (CNS) level that may be correlated with the degree of clinical disability assessed on Kurtzke's scale (EDSS). The optic nerve, as part of the central nervous system (CNS), can be relatively easy to evaluate and could provide the desired answer.

METHODS

Study population

We studied a batch of 111 patients, each diagnosed according to the criteria revised by McDonald (7), with multiple sclerosis of various types. For this batch we assessed the clinical disability on the EDSS scale, but also on sub-scales of Multiple Sclerosis Functional Composite (MSFC) such as time to walk 25 feet (TW25F) – for the evaluation of lower limbs disability – and Nine Hole Peg Test (9HPT) – for the evaluation of upper limbs disability.

The EDSS evaluation and MSFC quantification were performed by a neurologist and a psychiatrist having specific training for these types of evaluations.

Damage of the optic nerve was measured for each eye by: visual acuity (VA), P100 wave latency, retinal nerve fiber layer (RNFL) by OCT.

Clinical, electrophysiological and OCT evaluations were performed during an attack-free period for the patients with relapsing types of the disease.

All patients signed an informed consent to participate in the study.

Visual evoked potentials (VEP)

Visual evoked potentials are electrical potentials produced by visual stimuli and recorded on the scalp. This method tests the function of the visual pathway, from retina to occipital cortex, providing important diagnostic information about the integrity of the visual system.

Over 75% of multiple sclerosis patients exhibit abnormal values on VEP evaluation of optic nerve (VEP abnormalities are reported in percentage varying between 75-97%) (8, 9).

Visual evoked potentials reverse pattern have been used. They are represented by a series of positive and negative waves. The positive ones are denominated by the letter P followed by a number indicating the latency of the peak expressed in milliseconds (e.g. P60, P100) and the negative ones are denominated by N followed by a number indicating the latency of the peak in milliseconds (e.g. N70, N145).

VEP abnormalities could be transitory, meaning that the amplitude of the response can go back to normal in the same time with eyesight recovery, but the latency of the wave remains modified. This feature helps for retrospective diagnosis of a subclinical or previously ignored optic neuritis within the context of multiple sclerosis (8,10).

Electrophysiological evaluation was effected by a neurologist experienced in electrophysiology and respectively in VEP performance.

Retinal optical coherence tomography

Optical coherence tomography (OCT) quantifies mainly the retinal nerve layer as well as optic disk morphology. The abnormal values of the retinal fibers thickness were coded in yellow or red colors, depending on the degree of neuronal loss while the normal values are green.

In this study, the images of the optic nerve were taken and processed with the 3D system OCT 1000 TOPCON (OCT Software) which is an image system functioning with OCT for the purpose of posterior pole study. Type of scan: circular, scan surface: 2.4 mm after administration of 1 drop mydriatic substance (*Tropicamid* 1%).

The evaluation was performed by an ophthalmologist experienced in optic nerve evaluation by OCT.

By comparison with data present in optical coherence tomography software's database, the thickness of the retinal nerve fiber layer could be classified in normal (green), borderline (yellow-corresponding to percentile 5), and abnormal (red-corresponding to percentile 1). One measurement highlighted in red has a 1 percent chance to represent a normal RNFL value.

Statistic analysis

The results were uploaded into a database which was statistically analyzed using SPSS 17.0 software. Pearson type correlations were used for parametric variables, while for non-parametric variables we used chi square type correlations.

RESULTS

Characteristics of the studied population

Average age of the 111 patients participating in the study was 38.09 (between 16 and 60 years old) and the average EDSS score 3.3 (between 0.0 and 7.0). 76 out of the 111 patients were women.

The majority of the patients had the relapsing remitting type of the disease. The distribution according to the type of MS among the group is shown in the table 1.

TABLE 1

Clinical forms of MS	Number of patients	Percent
Relapsing Remitting MS (RR)	79	71.3%
Clinically Isolated Syndrome (CIS)	7	6.3%
Secondary Progressive MS (SP)	18	16.2%
Primary Progressive MS (PP)	6	5.4%
Primary Progressive with Relapses(PR)	1	0.9%
Total	111	100.0%

79 patients have the relapsing remitting type of disease (RR), 7 patients have the clinically isolated syndrome (CIS – the onset form), the rest of them presenting progressive types of MS: (18 – secondary progressive type and 7 primary progressive type, one of which showing the primary progressive type with relapse).

EDSS correlates statistically significant with the physical disability scores of MSFC

We have obtained statistically significant positive correlations between EDSS score and 9HPT score for the dominant hand ($r = 0.58$, $p = 0.0001$) and for the non-dominant hand using Pearson correlation.

A similar result was obtained by correlating the EDSS with the disability test for lower limbs, respectively TW25F ($r = 0.35$, $p = 0.0001$) via Pearson correlation.

EDSS correlates statistically significant with VEP and AV for both eyes

For each eye, we have correlated the visual acuity assessed on Snellen scale and the value of P100 wave latency obtained on VEP, with EDSS score.

We obtained statistically significant negative correlations between the visual acuity and EDSS: for the right eye (r = -0.45, p = 0.0001) and for the left eye (r = -0.49, p = 0.0001).

We also obtained statistically significant positive correlations between the value of P100 wave latency and EDSS score. For the right eye: (r = 0.405, p = 0.0001) and for the left eye: (r = 0.400, p = 0.0001).

Graphic representation type scatter plot of the correlations between EDSS and VEP, right eye and left eye is showed in figure 2a and b.

A subsidiary analysis was performed by arbitrary segregating the group into inflammatory and respectively progressive type of multiple sclerosis. 25 patients have progressive forms and the rest of 86 have inflammatory forms.

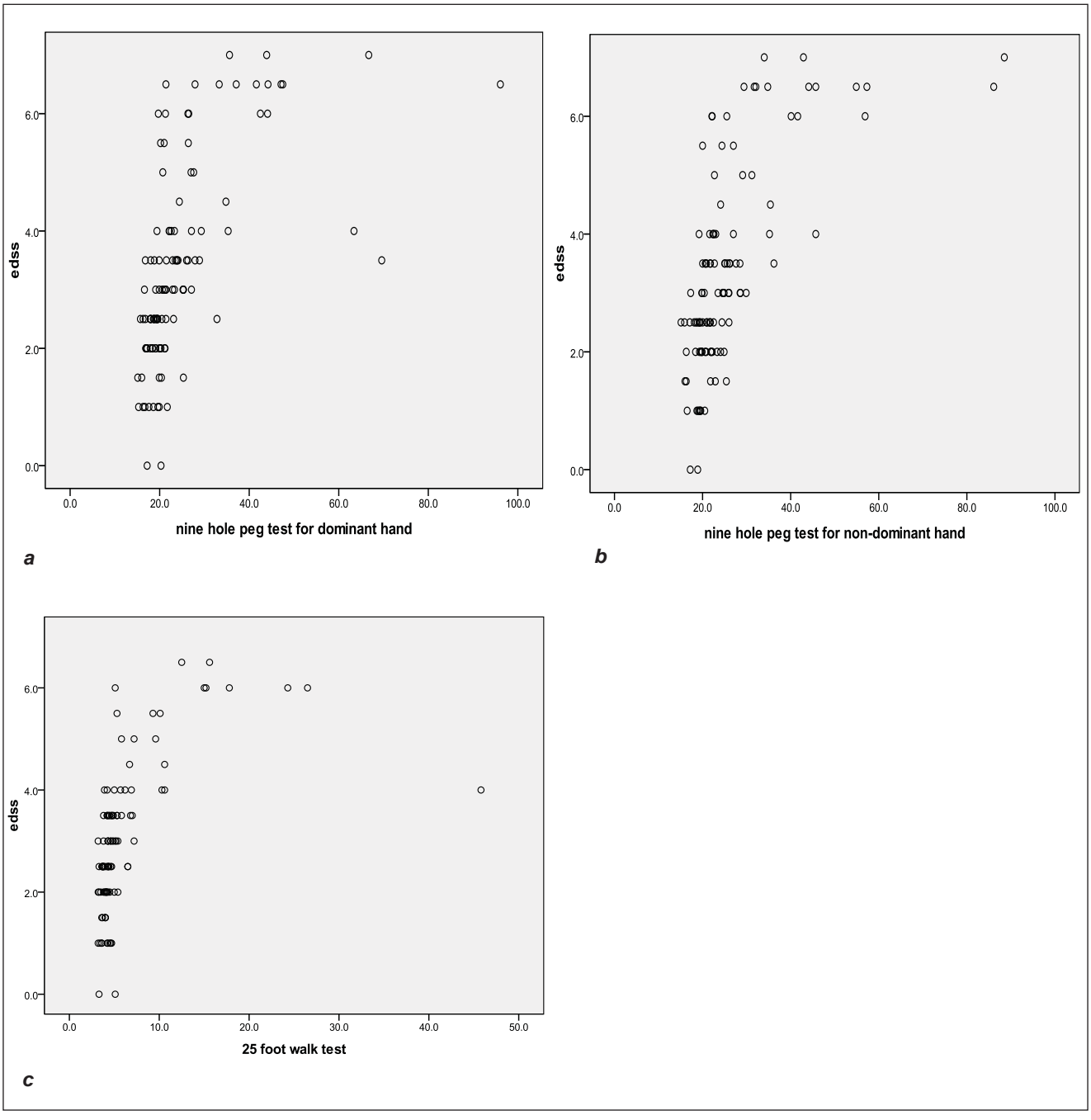

FIGURE 1 *a, b and c (graphic representation type scatter plot of correlations between EDSS and TW25F -1c- and 9HPT (dominant –1a and no-dominant hand-1b)*

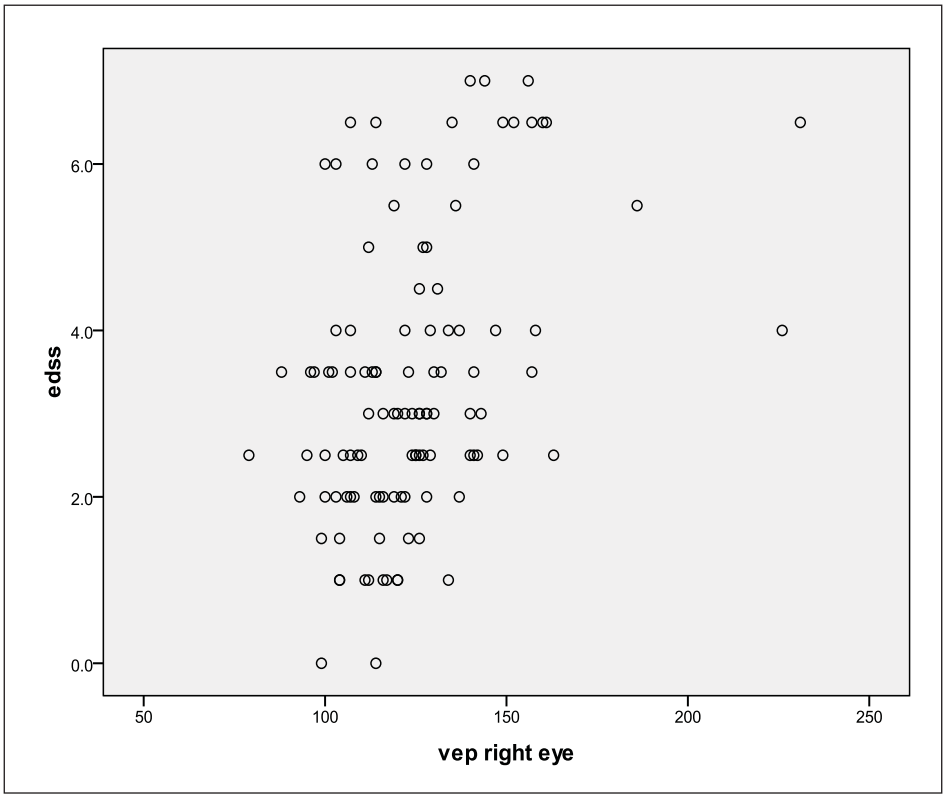

***FIGURE** 2a*

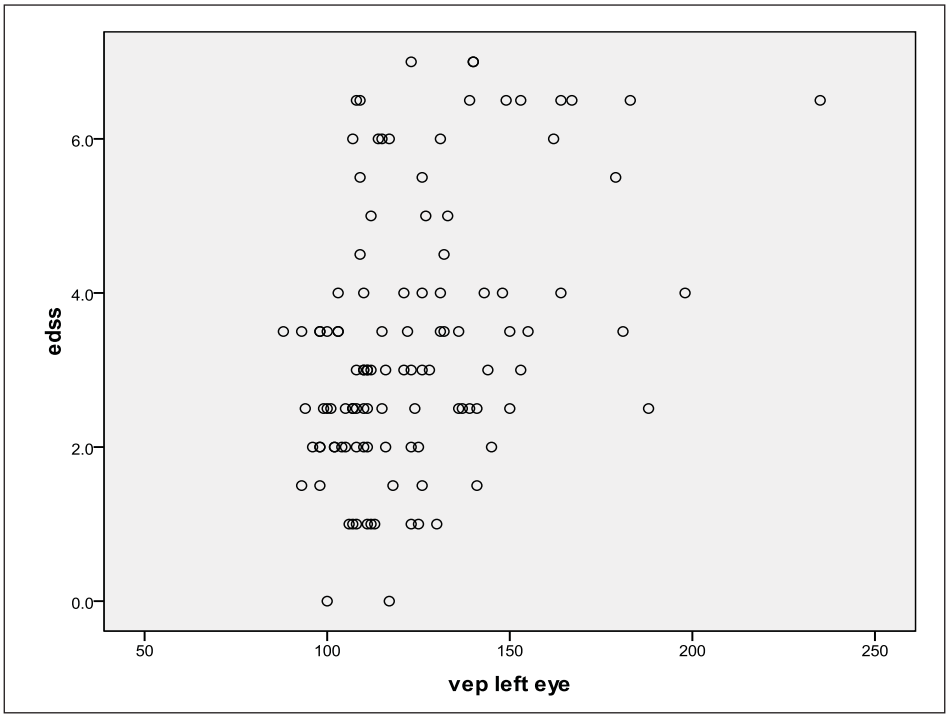

***FIGURE** 2b*

Functional tests for optic nerve evaluation, specifically visual acuity and P100 wave latency, have correlated for each eye with EDSS score, more clearly for the inflammatory types.

Clinical assessment of optic nerve, respectively the visual acuity, correlates with functional electro physiologic biomarker (P100 wave latency for each eye)

Pearson correlation between visual acuity (measured on the Snellen scale) and the P100 wave latency for each eye is statistically significant negative. Thus, for the right eye: ($r = -0.52$, $p = 0.0001$) and for the left eye: ($r = -0.44$, $p = 0.0001$).

Retinal fiber layer does not correlate either with EDSS, with the visual acuity or with P100 wave latency

The results obtained on assessment of retinal fiber layer were non-parametric; therefore the non-altered optic nerve was coded into green in all measured sectors of the optic papilla, while a focal loss in each retinal sector was coded into yellow or red by the OCT software. Yellow and red color codes are regarded as abnormal.

70 patients (140 eyes) were examined by OCT out of the total of 111 patients in the group.

Statistical correlations of chi square type have been made between the values obtained on OCT and the visual acuity (VA) for each eye, regarding the VA value of 1 as normal and lower values as abnormal. The results were not statistically relevant, presenting a low power of association: for right eye ($p = 0.7$, $phi = 0.1$) and for left eye ($p = 0.8$, $phi = 0.2$).

We have considered as normal values of P100 wave latency those equal or lower than 120 milliseconds, while higher values were regarded as abnormal.

No statistically significant correlations have been obtained for the left eye using the chi square method between the degree of retinal neuronal loss evaluated by OCT and the P100 wave latency determined by VEP ($p = 0.74$, $phi = 0.038$); however the values for the right eye had statistical significance ($p = 0.02$, $phi = -0.27$).

We set cut off values of EDSS that quantifies important physical disabilities on different neurological functional scales; thus, EDSS 3.0 and respectively 4.0 were taken into account for chi square type correlations.

Statistically significant correlations were obtained through this method, as well as through the Pearson linear correlation between EDSS (milestone of 3.0 and 4.0) and the values of visual acuity, and also between EDSS and the value of P100 wave latency for each eye.

We did not obtain statistically relevant results at chi square correlation between EDSS (cut off value 3.0) and the degree of retinal neuronal loss. The value obtained by OCT for the right eye: ($p=0.9$, $phi=0.01$) and for the left eye: ($p = 0.3$, $phi = -0.1$). Similar results were obtained for an EDSS cut off value of 4.0. For the right eye: ($p = 0.9$, $phi = -0.009$) and for the left eye: ($p = 0.8$, $phi = 0.017$).

DISCUSSION

For understanding the pathology of multiple sclerosis, the anterior optic pathway, and respectively the examination of optic nerves offer an attractive model. The relationship between demyelinating and neurodegenerative pathologies was not fully understood for this disease. A recent pathological postmortem study has performed a histopathologic analysis of optic nerves, starting at the retinal nerve layers and up to the chiasma, in search for an answer to this dilemma.

Alterations have been found at the level of retinal fiber layers, at the level of ganglion cells, but also at the level of internal nuclear layer, thus the lesions are more extensive than previously assumed. Although the retinal fiber layer is myelin free, it has been demonstrated the presence of inflammation at this level even in tardive stages of the disease (11).

Optic nerve assessment can be done by non-invasive methods from which we can obtain complementary information. Thus, by visual acuity measurement, visual evoked potentials performance and by retinal optical coherence tomography, important functional and structural data about the optic nerve can be obtained. All those tests together are able to identify the optic nerve abnormalities in multiple sclerosis in a 98 percentage. Visual acuity measurement (subjective method, based on patient's declarations) and visual evoked potentials (objective method) offer information regarding optic nerve axonal pathology, while the structural test (OCT) brings data about axonal loss (12,13).

The capability of VEP to detect the subclinic process of demyelination in the optic nerves brings information useful for the diagnostic process (space dissemination), but it can also be a tracking method for disease progression (12).

It was recently published a study that analyzes by OCT the retinal fiber layer in multiple sclerosis (14). It has been ascertained that retinal fiber loss may be encountered both in patients with remitting relapsing type of disease, and in case of those with

secondary progressive type, especially if their clinical history reports optic neuritis.

The retinal fiber loss could be generated by axonal degeneration secondary to focal inflammatory processes located distal on the optic pathway, but could also be caused by the diffuse axono-neurodegenerative progressive process. Therefore, it remains still unclear if the analysis of retinal nervous fiber layer (RNFL) represents a tracking method of disease progression (14,15).

The group under study is composed of patients with progressive type of disease (in which dominate the neurodegenerative processes) in a 22.5 percent, the rest having types of multiple sclerosis in which the inflammatory processes predominates (77.5%). Similar distribution is reported in epidemiologic studies.

The method currently used by clinicians to assess the physical disability (calculation of EDSS score) has correlated with the sub-scores of MSFC that reflects the physical disability of lower and upper limbs.

The data resulted from functional evaluation of optic nerves (visual acuity and P100 wave latency at VEP) have correlated statistically significant with each other (for each eye), but also with the physical disability score EDSS.

The assessment of retinal nervous fiber layer and of the axonal loss at the level of optic nerve did not correlate with the physical disability score.

It is a known that disability, in general, is given by the degree of axonal loss, but also that the axonal pathology is reflected by the disability score EDSS.

This study demonstrates that axonal pathology of the optic nerve (component of the central nervous system rich in white matter around axons) correlates with EDSS score (clinical marker of physical disability), suggesting that VEP (measurement of P100 wave latency) may be used as a functional biomarker for this disease. Based on these data a prospective study is needed in order to validate this marker for multiple sclerosis progression.

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